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A RAPID METHOD OF MARKING CORN EARWORM MOTHS WITH DYES FOR RELEASE STUDIES[1,2]

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A planned attempt to use the sterile male technique to eradicate the corn earworm, Heliothis zea (Boddie), from St. Croix, U.S. Virgin Islands, necessitated the development of a method to mark large numbers of moths.

Radioisotopes have marking potential, but they were not considered because of the radiation hazard associated with the daily treatment of many moths.

A device (moth marker-spray), using dye in an aerosol, was designed to rapidly treat large numbers of corn earworm moths without adversely affecting the insects' sexual behavior or creating hazards to the personnel involved. Crystal[5] used an aerosol-generating apparatus for treating screwworm flies with chemosterilants. Hathaway and coworkers used an aerosol applicator for applying tepa to codling moths for sterilization.

METHODS AND MATERIALS

The moth marker-spray is essentially a 7-inch-diameter metal pipe 36 inches long, with an aerosol generator (nozzle) in each end and means for placing moths inside the pipe. Pictorial views of the moth marker-spray with doors removed are shown in figure 1, A and C. For illustration, the moth marker-spray may be divided into three sections (A, B, and C) (fig. 1, C). Sections A and C are similar. Each is a 7-inch-diameter by 12-1/4-inch pipe with a nozzle in one end. The nozzle is a Spraying Systems® pneumatic atomizing nozzle siphon setup No. 1. It sprays a round pattern and requires 0.66 c.f.m. air at 20 p.s.i. Section B is also a 7-inch-diameter metal pipe placed inside a plywood drawer. When the drawer is closed the pipes of sections A, B, and C are alined. When the drawer is opened, moths may be placed inside for spraying or removed. The pipe is enclosed with 0.5-inch outside plywood that makes a unit 39 inches high by 13 inches wide by 15 inches deep. Cross-sectional drawings of the sections, showing details and dimensions, are shown in figures 2 and 3.

The air supply for the nozzles is a Dayton Speedaire® compressor No. 1Z629. Air to the nozzles is controlled by a solenoid valve. When a minimal air supply of 20 p.s.i. is available, a two-way solenoid valve would be satisfactory. However, our Dayton compressor has no unloader valve, therefore a three-way valve must be used to prevent possible damage to the compressor caused by overload when the nozzles are not spraying. An interval

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[1] Agricultural Engineering Research Division and Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, in cooperation with the University of Georgia, College of Agriculture, Coastal Plain Experiment Station, Tifton, Ga.

[2] Mention of a proprietary product does not necessarily imply its endorsement by the U.S. Department of Agriculture. ® indicates a registered trademark.

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[5] Crystal, M. M. Sexual sterilization of insects by aerosol administration of alkylating agents. Jour. Econ. Ent. 58(4): 678-680. 1965.

[6] Hathaway, D. O., Butt, B. A., and Lydin, L. V. Sterilization of codling moths by aerosol treatment with Tepa. Jour. Econ. Ent. 61(1): 322-323. 1968.

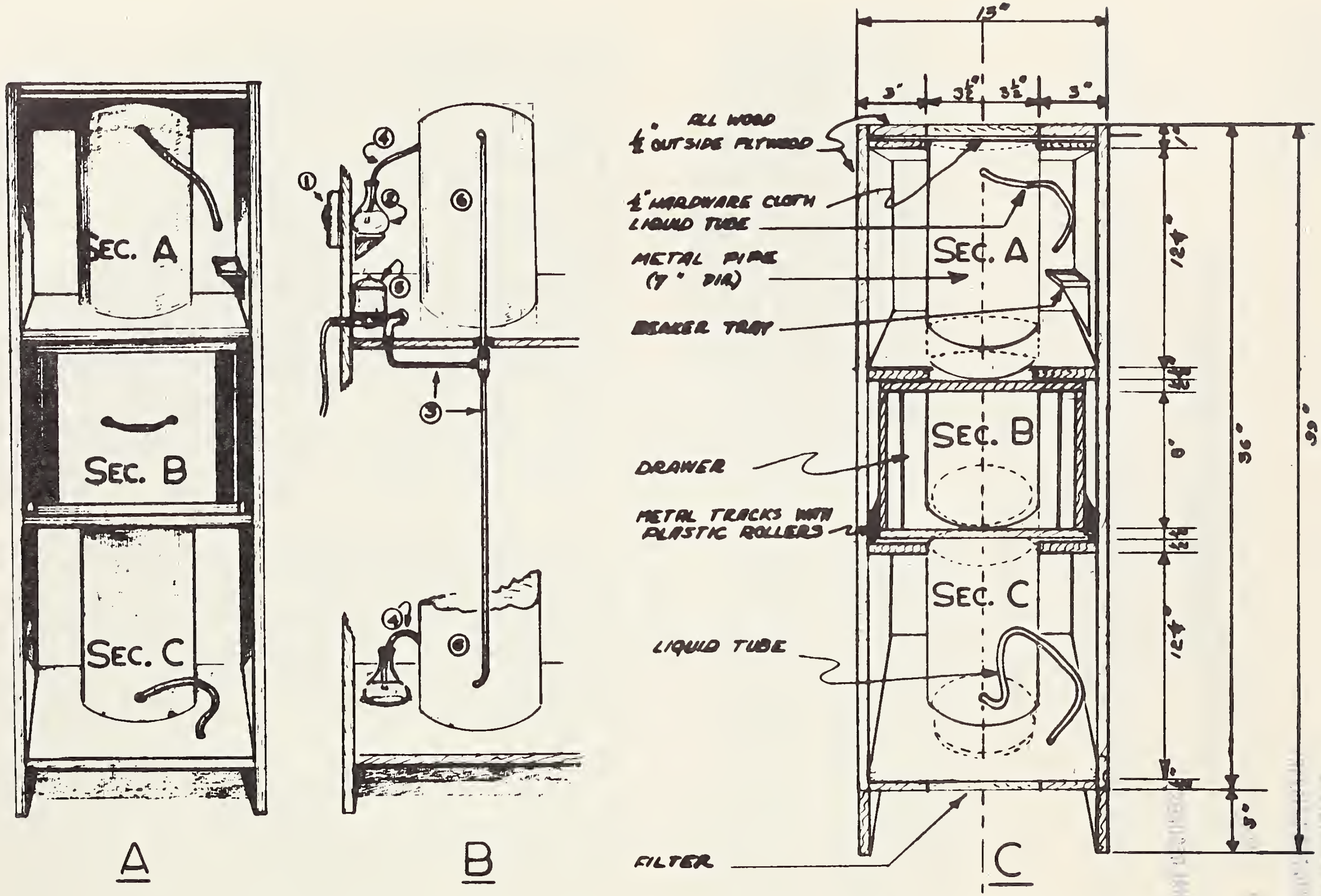

Figure 1.--Moth marker-spray: A, Front view, with upper and lower doors removed. B, Rear-view detail: meaning of circled numbers are: 1, Automatic timer; 2, dye reservoir; 3, air line (copper); 4, liquid line; 5, 3-way solenoid valve; 6, metal pipe (7-inch diameter). All wood 1/2-inch outside plywood. C, Front-view detail

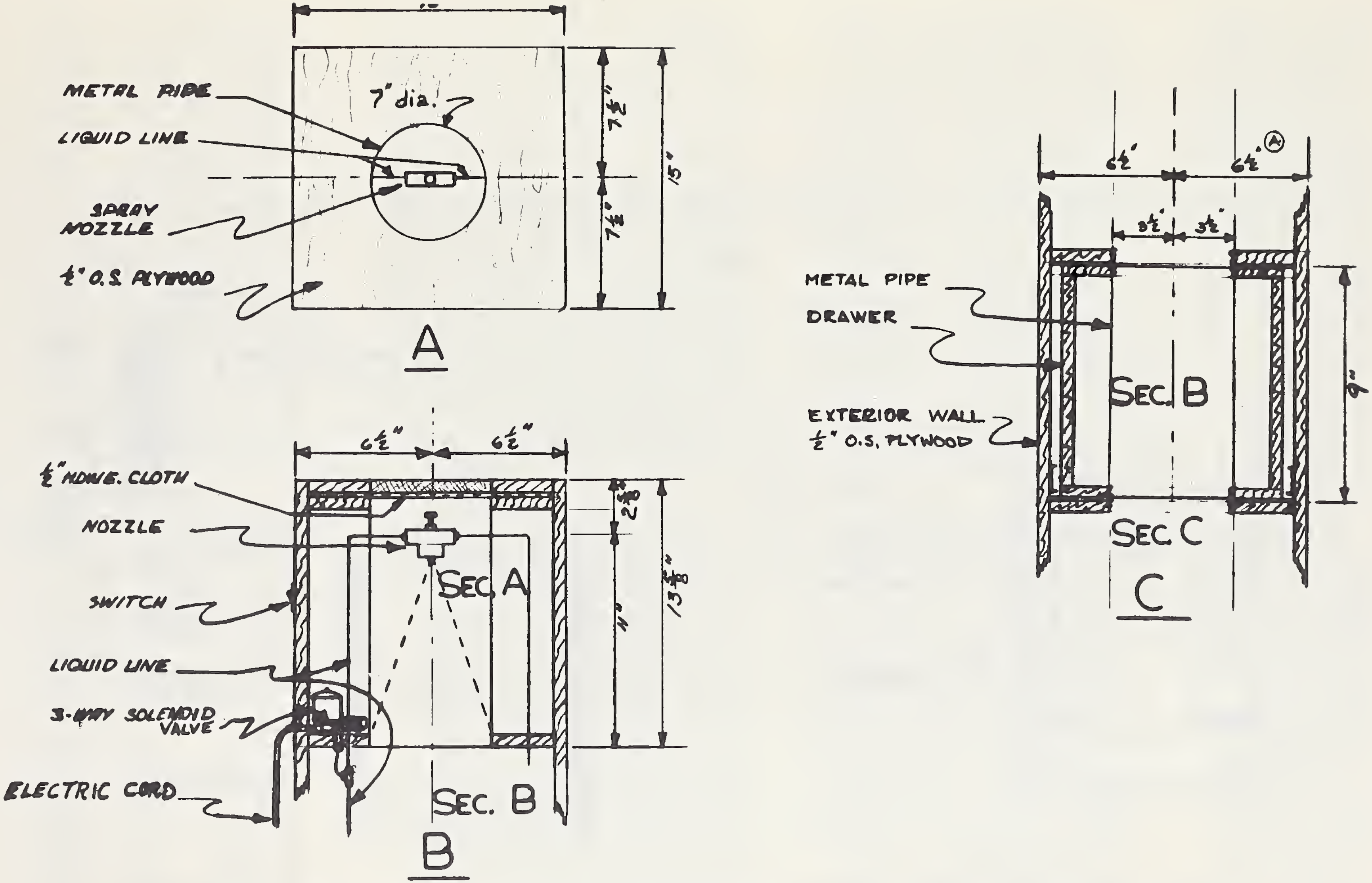

Figure 2.--A, Top view of top section; B, cross section of top section; C, cross section of section B, showing details of drawer.

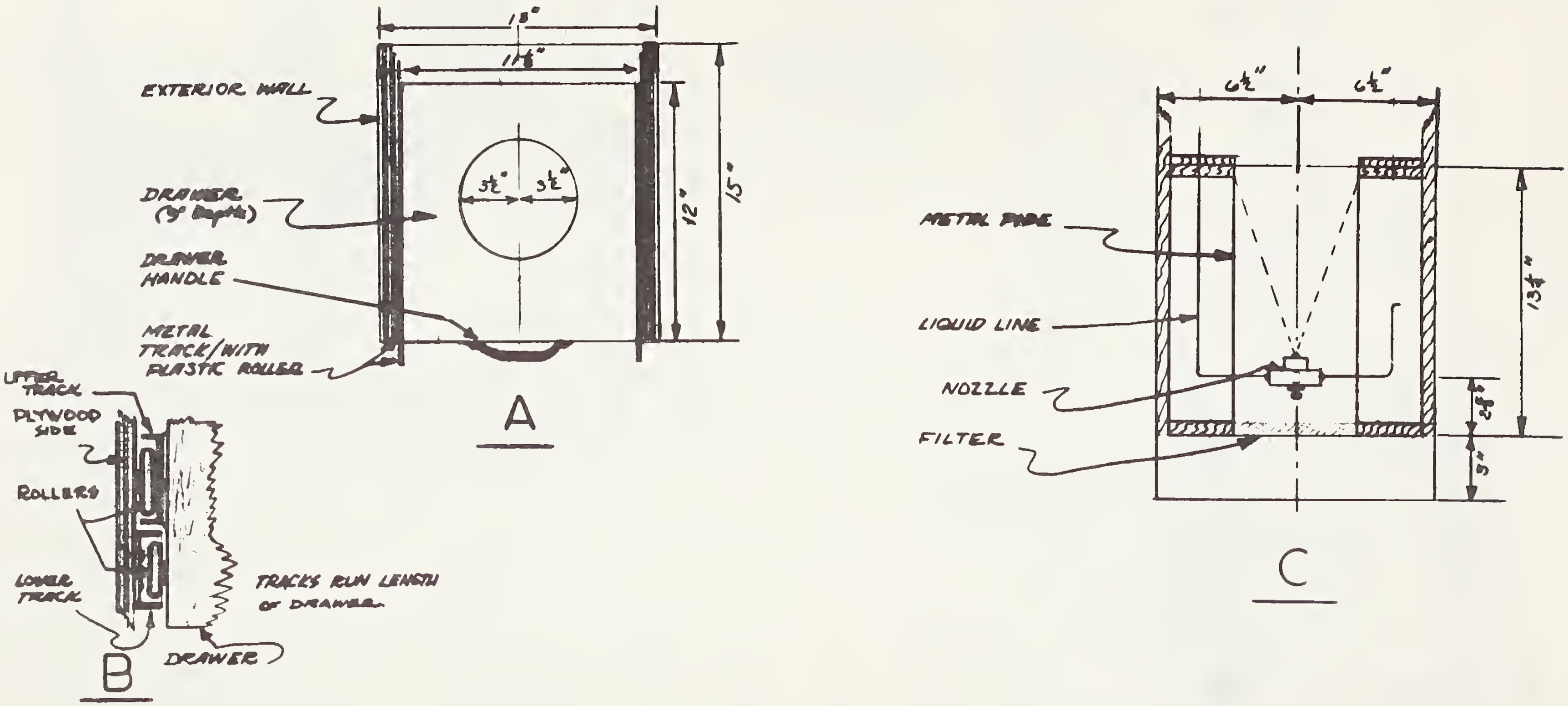

Figure 3.--A, Top view of section B; B, Detail of upper and lower tracks and rollers of drawer, left front view; C, cross section of section C.

timer in the solenoid electrical circuit controls the duration of the spray. A standard air-conditioning filter cut to size is placed over the top and bottom of the moth marker-spray to prevent spray drift. The moth marker-spray should be positioned over a drain that collects and removes excess spray.

The moth cages are 1-gallon ice cream cartons (6.8-inch diameter by 7 inches high[7]) covered on each end with a mesh cloth. The mesh cloth is Mag#72 obtained from Fablok Mills, Inc., Murray Hill, N.J. It is about 0.009 inch thick and has about 90 percent of its area open.

OPERATION

The moth marker-spray is located inside a chilled room (50° F.) to minimize moth flight. The moths cling to the walls of the cage. This results in all individuals receiving about the same treatment. Satisfactory marking may be obtained in ambient air condition by using adequate mixtures and moth exposure intervals.

Up to 500 moths are placed into the 1-gallon ice cream carton cages. The cages are placed inside the drawer (section B) and the drawer closed. The timer, which energizes the solenoid, is set for about 5 seconds, and the solenoid opens the air line from the compressor to the nozzles. The nozzles spray only when the solenoid valve is energized and a spray material is available in the reservoirs. The length of spraying may be altered to suit the degree of marking desired by changing the interval on the timer. After the moths have been exposed to the spray for the desired interval, the drawer is opened and the cage removed. The moth marker-spray is then ready for the next cage of moths.

DYE SELECTION

Depending on solubilities, solutions of acetone, water (containing 500 p.p.m. of Tween 100®), or alcohol of various concentrations of Rhodamine B (C.I.[8] 45170), Rhodamine 6G (C.I. 45160), Rocket Red (Switzer Brothers, Inc., Cleveland, Ohio), Eosin Y (C.I. 45380), New Fuchian (C.I. 42520), Procion Brilliant Red 8BS (Arnold, Hoffman & Co., Providence 1, R.I.), Fluorescein Sodium (C.I. 45350), Sudan II (C.I. 12140), Oil Red O (C.I. 26125), and Solophenyl Turquoise Blue (Geigy, Ardsley, N.Y.) were tested. These represented fluorescent and visible markers. Treated moths were caged in 1-gallon ice cream cartons for 5 days; they were disturbed often to cause excessive movement and were treated with simulated rain to ascertain those dyes that were retained the best. Other treated specimens were killed and pinned on boards and exposed to the sun rays. Several of the dyes showed great potential as a marker. However, Rhodamine B in a 0.1 percent water or acetone solution was superior as a visible marker. Henneberry and coworkers[9] also used 0.1 percent Rhodamine B as a marker for the cabbage looper. For these reasons, subsequent mating experiments were conducted with this dye.

EXPERIMENTAL MATING

A series of tests was conducted to determine if mating was affected by the Rhodamine B treatment of the moths. Both sexes were tested even though females will not be released. Dissection of spermatophores from the corpus bursa was the criterion for determining mating status.

Experiment 1.--Ten female moths caged with normal males or Rhodamine B treated males were placed in 1-gallon ice cream carton cage and fed a beer solution. After 5 days, females were dissected. Results of this series of tests are shown in table 1. Nonsignificant differences in mating were observed between

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[7] Snow, J. W. A holding cage and handling device for noctuid moths. Jour. Econ. Ent. 59(6): 1547-1548. 1966.

[8] C.I., color index.

[9] Henneberry, T. J., Howland, A. F., and Wolf, W. W. Recovery of released male cabbage looper moths in traps equipped with blacklight lamps and baited with virgin females. Jour. Econ. Ent. 60(2): 532-537. 1967.

Table 1.--Numbers of spermatophores dissected from 10 females per replicate caged for 5 days with 10 Rhodamine B treated or 10 normal males per replicate, Tifton, Ga., 1968

Replicate No.	Number of spermatophores from females caged with--	
	Normal males	Treated males
1	8	6
2	6	7
3	7	5
4	7	7
5	7	8
6	6	14
7	7	7
8	8	7
9	11	12
10	11	9
Average	7.8	[1]8.2

[1] No significant difference from the normal.

the treatments. In the closely confined quarters of the gallon cage, no disadvantage due to treatment of moths was observed.

Experiment 2.--In a large (12-X12-foot) mating chamber provided with growing corn plants in pots, 10 normal females and 10 Rhodamine B treated females were released with 20 normal males. The females were dissected after 5 days. The experiment was replicated four times. In a cage this size some orienting behavior is necessary for the sexes to find each other; therefore, detrimental effects of the dye could be observed because of the probable decrease in random mating. Differences in numbers of spermatophores per female were nonsignificant (table 2).

Table 2.--Numbers of spermatophores dissected from 10 normal and 10 Rhodamine B treated females per replicate caged with 20 normal males, Tifton, Ga., 1968

Replicate No.	Number of spermatophore from females	
	Treated	Untreated
1	7	6
2	7	12
3	9	7
4	10	11
Average	8.3	[1]9.0

[1] No significant difference from the normal.

CONCLUSION

Rhodamine B satisfactorily marks the corn earworm for identification of marked release insects. No decrease in mating frequency indicates no significant biological effects to the moth. Thus, the described moth marker-spray system seems adequate for rapid treatment of large numbers of moths for release programs.

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